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Review

Physiological and Biochemical Basis of Faba Bean Breeding for Drought Adaptation—A Review

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Abstract: Grain legumes are commonly used for food and feed all over the world and are the main source of protein for over a billion people worldwide, but their production is at risk from climate change. Water deficit and heat stress both significantly reduce the yield of grain legumes, and the faba bean is considered particularly susceptible. The genetic improvement of faba bean for drought adaptation (water deficit tolerance) by conventional methods and molecular breeding is time-consuming and laborious, since it depends mainly on selection and adaptation in multiple sites. The lack of high-throughput screening methodology and low heritability of advantageous traits under environmental stress challenge breeding progress. Alternatively, selection based on secondary characters in a controlled environment followed by field trials is successful in some crops, including faba beans. In general, measured features related to drought adaptation are shoot and root morphology, stomatal characteristics, osmotic adjustment and the efficiency of water use. Here, we focus on the current knowledge of biochemical and physiological markers for legume improvement that can be incorporated into faba bean breeding programs for drought adaptation.

Keywords: *Vicia faba*; drought; root; shoot; metabolomics

1. Introduction

Among the cultivated grain legume crops, the faba bean (*Vicia faba* L.) ranked sixth globally in production, with a value of 4.5 Million tonnes from 2.5 Mha in 2019 [1]. The common bean (*Phaseolus vulgaris* L.) ranked first, followed by the field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* (L.) Walp) and lentil (*Lens culinaris* Medik.), and the global average yield per hectare of the faba bean exceeds all of these other species. The faba bean was one of the first domesticated crops in the Middle East [2] and spread from there eastward to Central Asia, India and China; westward to North Africa and Europe and, in more recent times, to Australia and the Americas [3]. While the wild antecedent of the faba bean is not known [4], carbonised remains dating back to 14,000 B.P. are viewed as examples of its existence [5].

Grain legumes provide substantial advantages in cereal-based cropping systems by breaking the life cycles of certain diseases, host-specific nematodes and weed species [6]. Like other grain legumes, faba bean and its symbiotic rhizobacteria fix atmospheric nitrogen in a wide range of conditions. Biological nitrogen fixation provides about 80% of the plant's nitrogen needs [7], reaching 160 kg ha^{-1} [7–9], and about half of the crop's fixed nitrogen content is left in the field after grain harvest. Hence, it is considered important for both its contribution to residual nitrogen in crop rotation [6] and its potential in green manuring [10].

Faba bean seeds have high contents of protein and micronutrients and are also a good source of energy and dietary fibre [11,12]. The average protein content is 29% [13], ranging from 24% to 35% [14], making it one of the most protein-rich pulses (starchy grain legumes). Mature beans are cooked into a stew or paste for human consumption in many countries and are also used as animal feed in many others. In its vegetable form, immature pods are harvested when the seeds are filling, and the young seeds are used as salad or cooked as vegetables. Whole-crop silage can be made from the whole plant and is a way of rescuing value from the crop if, for example, an exceptionally wet autumn prevents a harvest of the dry grain.

Among cultivated grain legumes, the faba bean is considered to be sensitive to water deficit (Figure 1). The faba bean yield in irrigated agriculture is significantly higher than in rainfed systems [15], although the majority of its cultivation lies outside irrigated areas. Yield-based meta-analyses data from 1980 to 2014 indicated that the faba bean yield was reduced by 40% following a 65% reduction in water availability, and the loss in yield depended on the cultivar and other environmental conditions [16]. Improving drought tolerance is vital to improve the performance and stability of the yield, but efficient selection protocols and the mixed breeding system of this crop pose major challenges for effective plant breeding programs [17,18]. Field phenotyping for drought responses is expensive and time-consuming and is often unrepeatable because of variations in the timing of onset, duration and severity of the drought [19]. The mean outcrossing rate is about 33%, depending on the population of pollinators, cultivars and environmental conditions [20], and if cross-pollination between susceptible and tolerant plants occurs before drought stress occurs, the effectiveness of selection for the drought response is compromised [21]. On the other hand, controlled condition screening based on secondary physiological characteristics of roots and shoots, including stomatal conductance, relative water content, water potential, osmotic potential, root morphology, root plasticity and isotopic carbon discrimination, can provide generally reliable and repeatable information for selection, but the population size is compromised. Metabolic profiling, which provides insight into how changes in metabolite concentrations are influenced by changes in the environment, also holds great promise. In this review, we bring together the current physiological and biochemical knowledge on traits related to drought adaptation in the faba bean and elaborate how this knowledge can be used in breeding faba beans. A schematic overview of the roadmap to achieving these aims and how these relate to one another is shown in Figure 2.

Figure 1. Faba bean plants (FB 9-4) under water-deficit conditions (**left**) and irrigated conditions (**right**) in Limerick and Outlook, Saskatchewan (Canada), respectively.

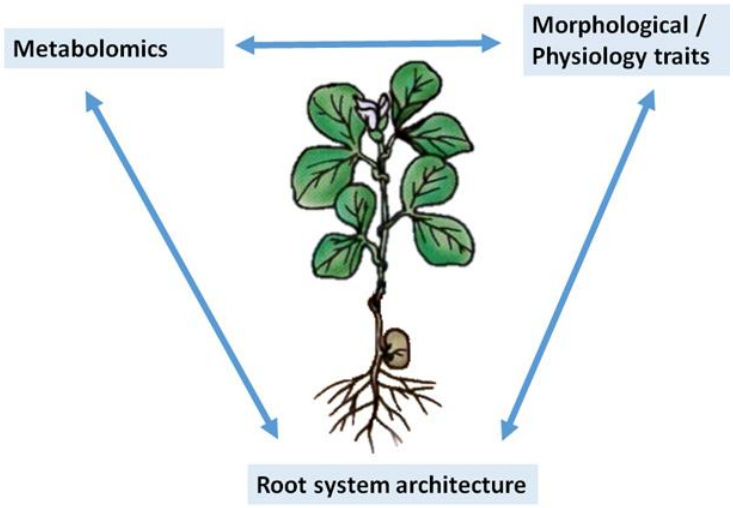

Figure 2. The key elements of the roadmap and how these relate to one another.

1.1. Faba Bean Yield Response to Drought

Three types of strategies to cope with drought stress have been characterised—namely, drought avoidance, drought dehydration tolerance and drought escape. Drought avoidance refers to mechanisms related to keeping a high plant water status under water-limited conditions by minimising the water loss and maximising the water uptake through root characteristics. Dehydration tolerance refers to maintaining metabolic activities at the low-tissue water potential (surviving internal water deficits). Drought escape is the ability of a crop to complete its life cycle before the onset of drought or unfavourable conditions [22]. The faba bean is prone to water deficit from seedling to maturity [19,22], but in the Mediterranean-type climates, where it is sown in autumn and harvested in spring as the temperature increases and rainfall declines, the faba bean often experiences so-called "terminal" droughts [23]. Adapted materials escape terminal droughts by early maturity, completing their life

cycles before a drought occurs, often accompanied by a yield penalty. Besides terminal drought, the faba bean often experiences transient droughts in subtropical and temperate environments. Transient droughts reduce the plant stature and number of reproductive nodes in faba beans [24]. In contrast to escaping, there is no comparable and simple strategy to deal with unpredictable and intermittent transient droughts. Maintaining higher relative water contents under water deficits, which demonstrates effective stomatal regulation and stomatal function [25], root morphology, plasticity and function [26], epicuticular wax and osmotic adjustment [22], play vital roles in the management of transient droughts.

Although the faba bean originates from a semi-arid region, drought adaptation is not common. Its relatively shallow root system [27,28] is unable to extract water from deep soil, causing moisture stress during the reproductive phase when the topsoil dries. Genetic resources that can adjust to variations in soil moisture levels exist [19,29–33], indicating that there is potential to breed for moisture-limited environments. Link et al. [32] selected drought-tolerant cultivars and breeding lines from the Mediterranean region where water distribution is irregular and moisture levels are moderate (~500 mm rainfall). Maalouf et al. [34] also found different tolerance levels to water deficits. The existence of wide genotypic variations makes it possible to develop drought-tolerant faba bean genotypes through classical breeding approaches.

The plant developmental stage and the magnitude of water deficit determine the yield loss of the faba bean. The most susceptible stages for developmental inhibition have been variously described as flowering [35], early podding [36] and pod setting [37], but all of these studies generally agree that the early reproductive phase is the most sensitive stage [19,38]. Moderate drought stress had a negative effect on the pod number per plant but had no effect on the seed size or seed number per pod [35,39]. The extent of drought stress determined whether the plant had partial damage or complete death. These observations highlight the importance of identifying the developmental stage when assessing the impact of drought conditions in faba beans.

Seed quality is also affected by water deficit. Genotypic background and environmental factors, i.e., drought and heat, are the major factors that regulate seed protein contents [40]. Winter faba beans have slightly higher protein concentrations than spring beans, which may be interpreted as an indication that water deficits tend to increase protein contents [41]. Faba bean crops grown in water-deficit conditions have improved proteins [42] and sulphur contents [43] compared to those grown with adequate water. In drought-prone environments, reduced protein biosynthesis is due to reducing the N fixation and partitioning [44]. On the other hand, Smith [45] reported that droughts caused yield reductions but did not alter the mineral nutritional quality (calcium, iron, potassium, magnesium, phosphorus, sulphur and zinc) or amino acid concentrations in the common bean. Generally, seed mineral uptake is not impacted by drought. A high N fixation is usually observed in the faba bean under optimal conditions, whereas droughts can considerably impair the N fixation [46,47]. Under water deficits, modifications of the bacteroid environment cause the decline in N fixation [48] and reduced nodule numbers in faba beans [49].

Combined, these observations illustrate the importance of physiological responses to droughts in the faba bean and the influence this has on crop yields and yield quality. It is therefore important to identify tools by which the physiological status may be quantified to assist in management and breeding to help mitigate the effects of drought conditions on plant performances.

1.2. Physiological Attributes Related to Stress Adaptation

It is well-established that the phenotypic selection, based on adaptive traits that are secondary to yield, can be used for improving production in water-limited environments [50–52]. Secondary traits chosen for this purpose should be genetically associated with yield in the target environment, highly heritable and easy to measure. Drought-tolerance trait selections were found to be more meaningful when conducted on a large population [53]. Several physiological traits, including water use efficiency (WUE) [31], stomatal features [54–56], leaf temperature and carbon isotope discrimination [19], have

been found to be suitable selection criterion for the improvement of drought adaptation in faba bean breeding programs.

Drought stress interacts with low temperatures, soil salinity and Al^{3+} toxicity. Salinity and drought affect the osmotic equilibrium in a similar way [57], while Al^{3+} toxicity hampers root development [58], and cold affects both the movement of water within roots and the ability of the plant to develop symbiosis [59]. Among legumes, Al-drought interactions have been studied in the common bean [60] and soybean (*Glycine max* (L.) Merr.) [61], and the Al^{3+} toxicity exacerbated the effects of the drought mainly due to its inhibition of root growth. On the other hand, the drought was reported to ameliorate Al^{3+} injury by reducing the uptake of the toxic ion. The Al-drought interaction has a synergistic effect on crops, rendering them unable to recover after exposure to these stresses. Salinity coupled with drought reduced the symbiotic nitrogen fixation, which may be due to its effect on the supply of photosynthates or oxygen to the nodule and bacteroids [62]. Crosstalk in stress signalling and gene expression has been reviewed in drought and cold stress responses [63] and in cold and salinity responses [57]. In the faba bean, little genetic connection between the drought response and freezing tolerance was observed [64].

2. Root Traits

Compared with the aboveground traits, relatively few studies have been performed on belowground traits. A root trait analysis is complex, involving clumsy measurement systems, so maintaining a high level of precision is difficult in field level screening [65]. Among the root architectural features, morphology and plasticity are the two general types of variation that exist. Genetic variations in root morphological characteristics have a significant influence on the capacity and efficacy of a plant to search for and absorb moisture and nutrients from the soil. Deep-rooted soybean genotypes have a higher water-absorption capacity from deep horizons of soil, leading to a higher yield potential [66]. Moreover, the alteration of the root architecture (plasticity) follows several mechanisms under stress and nutrient availability. For example, the root/shoot biomass ratio increased in white lupin (*Lupinus albus* L.) and Andean lupin (*L. mutabilis* Sweet) during water deficit [67]. Changes in the root density of *L. angustifolius* genotypes in response to phosphorus availability have also been observed [68]. Faba bean genotypes with extensive and prolific root characteristics usually exhibit drought tolerance [29]. A variability in the root characteristics and root-based phenotyping for drought tolerance was also found in cowpeas [69], chickpeas [65,70,71], lentils [72,73] and the common bean [74].

Under water-deficit conditions, Husain et al. [75] showed an increased root growth per unit area during the flowering stage. Manschadi et al. [27] described the root length density, along with the dry root weight, and both were considerably reduced under drought conditions. Belachew et al. [26] identified root phenotypic markers associated with drought avoidance characteristics in young faba bean plants in GROWSCREEN-Rhizo boxes, a phenotyping robotic tool for measuring root and shoot growths [76]. Accordingly, two faba bean accessions (IG 70622 and IG 11320) that exhibit deeper root systems and higher root area coverage, displayed drought-avoidance traits through the maintenance of their primary and tertiary root lengths relatively well. The same observation was noted by Muktadir et al. [77] while screening drought-adapted genotypes in a hydroponic screening system. The growth of the tap root in the faba bean is a major influence on the total rooting depth; however, the tap root appears unable to acquire water for growth, even if the root surface has access to it. At shallow depths, lateral root growth is important for access to surface moisture and nutrients and is thought to be a major influence on plant health. Selection on the basis of rapid growth of the taproot and expansion of the lateral root system to maintain the root length density and dry root weight may be an effective method for increasing drought adaptation in faba bean breeding programs. There has been limited research on the genetic variability of root growth and branching in faba beans. Appropriate measurements of root systems include root mass, root mass fraction, rooting volume, root length and

root/shoot ratio. Among them, the root mass is probably the easiest to measure, as, in chickpeas, it was strongly correlated to the shoot mass and represented 30–34% of the total dry matter [65].

3. Shoot Related Traits

Under stress conditions, selection for a higher yield is not always effective, because yield is controlled by several traits controlled by quantitative trait loci (QTL). To select genotypes for drought tolerance, the breeding process should be focused on the contributing characteristics rather than the yield itself. Contributing characters need to be causal, and shoot-related traits play a vital role in maintaining the water status when grown in water-deficit conditions.

3.1. Leaf/Canopy Temperature

The temperature of the exterior of the canopy is related to the vapour pressure deficit and directly influences the amount of transpiration that leads to cooling by evaporation. Direct and easy measurement of the canopy temperature (CT) without disturbing the crop can be completed by using an infrared thermometer (IRT), which can be mounted on a drone to cover a large area in a relatively short time to minimise environmental fluctuations. Blum et al. [78] introduced CT as a selection trait for dehydration tolerance. During water-deficit conditions, closed stomata cause a decrease in transpiration, which ultimately leads to an increase in leaf temperature [79,80]. In field conditions, maintaining a lower CT is generally linked with improving the absorption capacity of a plant under stress conditions [81]. CT can be used as a substitute selection trait to screen faba bean genotypes grown in control environments [19,22]. In a large set of faba bean germplasm collected from regions with contrasting moisture regimes around the globe, the leaflet and canopy temperatures were the most informative measurements distinguishing the germplasm from wet and dry origins [82,83]. CT has been found to be a prominent trait associated with drought tolerance for soybeans [84], chickpeas [85–87], cowpeas [88] and lentils [89]. The preference for use of CT is its noninvasive nature and application to early evaluations in large numbers of genotypes. The protocol for using an IRT should be followed carefully to avoid a large variance error and nonrepeatable results. Thermal infrared imaging of crop canopies is a further technology for the study of stomatal responses and for phenotyping plants for differences in stomatal behaviours. The use of remote imaging, when combined with an effective image analysis tool, provides a powerful approach for the comparison of large germplasm collections in typical field conditions [90].

3.2. Leaf Water Relations

During water-deficit conditions, plants may maintain the water potential by the production and accumulation of cellular metabolites/osmotica, which are sensed by stomata for the maintenance of sustaining the turgor pressure. Plant species and their developmental stages are the determinants of the types and functions of these metabolites. Relatively few candidate molecules can function as cellular osmotica, as they must be compatible with cellular functions at high concentrations. These candidate molecules may be organic acids, inorganic ions, polyols, polyamines and carbohydrates. In chickpeas, carbohydrates and polyols contribute more than 50% to the osmotic pool [23], and in field peas, these ranged from 10–46% [91]. More broadly, the osmotic potential (ψs) and turgor potential (ψp), the two main components of leaf water potential (ψ), were found to be an effective selection trait for drought tolerance screening. Generally, leaf ψ is considered a major component of the leaf-level water deficit [92], and it can ultimately drive interpretations of the plant water status [93,94]. Sustaining leaf ψ under stress involves a number of mechanisms that are related to both root and shoot and are considered to be connected to drought avoidance [95]. Higher leaf turgor in response to water deficit is one of the adaptation strategies for many plant species [96,97]. Osmotic adjustment-driven drought adaptation was also observed in chickpeas [98,99], field peas [100] and lentils [101,102]. In faba beans, drought tolerance through osmotic adjustment has not yet been demonstrated in the wide germplasm but, as a selection criterion under drought, is sporadically observed, e.g., [103]. Metabolic adjustments

in response to drought conditions may highlight pools of metabolites that play vital roles in the metabolism and physiology and may indicate which pathways have been perturbed by the stress.

3.3. Relative Water Content

The relative water content (RWC) is a robust and simply assessable selection criterion that can describe the plant water status to the metabolism irrespective of plant parts and species. It can be expressed as the water content of tissues in normal conditions compared to hydrated conditions [104]. During water-deficit conditions, the RWC plays an important role by preserving water (stomatal features, leaf area reduction and leaves dropping) or maximising the water absorption (root plasticity). Sinclair and Ludlow [105] found the RWC superior over water potential to assess plant water statuses. Khazaei et al. [55] stated the RWC as one of the most important traits that differed between wet- and dry-adapted faba bean accessions under nonstress conditions. The RWC is composed of a relative change in cell turgor and ψs, so it relates to both the solute concentrations and cell wall rigidity [106]. The RWC can efficiently identify drought-tolerant genotypes based on their plant water status in faba beans [19,82,107], common beans [108] and chickpeas [109,110]. Therefore, it can be said that genotypes that can sustain a higher RWC in a water deficit environment would be suitable for use in breeding faba beans for drought adaptation.

3.4. Stomatal Conductance

Drought avoidance involves the maintenance of gas exchange during drought conditions, which causes either higher stomatal conductance, g_s (low stomatal sensitivity to drought) or stomatal closure to restrict water loss. There is a trade-off between gas exchange and stomatal closure during stress, which ultimately affects photosynthesis and, then, carbon assimilation. Stomatal conductance has been proposed as an effective selection tool and, when measured on multiple plants in a canopy, is equally effective as CT [111]. The measurement of g_s depends on several stomatal features. Faba bean stomatal characteristics are assessed through microscopy [25,29], but due to the rapid response to stress, the stomatal opening size is hard to measure. Leaf porometer or portable photosynthesis machines quantify g_s but may not reflect the variability over longer timeframes, as it changes rapidly. The measurement of g_s is highly influenced by environmental conditions (high genotype by environment interaction), time of day and plant developmental stage. Leport et al. [101] found that white lupin, chickpeas, faba beans, field peas, grass peas (*Lathyrus sativus* L.) and lentils all responded similarly under water-deficit conditions. During early stage drought, g_s was remarkably reduced, but at later stages, after the initiation of pod filling, it had little or no effect. Faba bean genotypes with higher stomatal density were lower-yielding and exhibited low drought tolerance, while genotypes with low stomatal density performed better in stress conditions [56]. In a relatively small set of faba bean germplasm, g_s was found to be an important vital trait [19,112]. The considerable variation for g_s among 402 faba bean accessions under nonstress conditions indicates the potential use of this trait [25]. The drought treatment of faba beans during the first three weeks of growth resulted in the expected reductions in biomass and g_s [113]. The gas exchange measurements, along with stomatal morphology characteristics, have been genetically mapped in the faba bean. The QTL governing stomatal morphology, g_s and CT were all co-located in faba bean chromosome 2 [83]. A study of the gene actions showed that breeders selecting for the productivity of faba beans can maximise genetic gain by selecting early generations for CT and g_s [114].

The effectiveness of stomatal control over transpiration efficiency increases if nonstomatal transpiration pathways are restricted. An important nonstomatal water vapour pathway is the leaf cuticle. The epicuticular wax (ECW) quantity is considered as an important drought avoidance strategy in plant species. There was a considerable variation for ECW in 197 faba bean accessions, ranging from 0.680 to 2.104 mg/dm^2 [115]. This variation may allow the genetic improvement of ECW as a drought-adaptive character in faba bean breeding programs.

3.5. Carbon Isotope Discrimination

Carbon isotope discrimination (Δ) is proposed extensively as a proxy for the plant physiological status, especially WUE [116]. It is used to evaluate the transpiration efficiency (TE) in cereals and, more recently, in legumes, common beans [117–119], soybeans [120], chickpeas [121], cowpeas [122] and lentils [123]. In faba beans, drought-tolerant genotypes ILB 938 and Mélodie had significantly lower Δ than those of the drought-susceptible genotypes, e.g., Aurora [19]. In a larger faba bean germplasm, Δ varied from 24.43% to 28.82‰, equivalent to an intrinsic water use efficiency (WUE_i) of 98.53–179.75 μmol CO_2 mol^{-1} H_2O (manuscript in preparation). There is some controversy regarding the relationship between Δ and TE. Turner et al. [123] argued that TE was not significantly correlated with Δ in three grain legumes, i.e., lentils, narrow-leafed lupin (*L. angustifolius* L.) and chickpeas. Carbon isotope discrimination may not always indicate the prediction of actual WUE, because generally, Δ is measured from leaf tissue, which has limitations for estimating short-term environmental changes [124]. Carbon isotope discrimination-based screening methods are expensive and require sophisticated and costly instrumentation, e.g., isotope ratio mass spectrometry (IRMS). A wider spectrum of faba bean germplasm needs to be surveyed for genetic variations in Δ under various environmental conditions to allow Δ to be used as a strong screening tool in faba breeding programs.

4. Metabolomics for Legume Breeding

Metabolomics is a fast-emerging area of knowledge that integrates gene expression, protein interaction and other regulatory progressions. Nowadays, metabolic products are used as biomarkers in crop improvement due to their strong linkage with environmental characteristics [125]. So far, more than 200,000 metabolites have been identified in plants. These have a diverse set of functions and quantification, and the qualification of these composite characteristics into subsets of metabolic functions can offer a unique view of the inducible chemical and physiological response mechanisms [126]. The identification and quantification of low molecular weight metabolites require special analytical and separation techniques involving gas chromatography (GC) [127], liquid chromatography (LC) [127,128] and high-performance liquid chromatography (HPLC) [129]. These methods can characterise a vast range of metabolites at extraordinary scales and accuracy. Recently, nuclear magnetic resonance (NMR) has provided a greater ability for researchers to profile metabolites [130].

Metabolic profiling studies of grain legumes are relatively unexplored and seemingly absent for faba beans. Zhang et al. [131] studied metabolic variations between symbionts in *Medicago truncatula* Gaertn. through an untargeted quantitative mass spectrometry-based (MS) method. Short-term water deficit effects on the overall growth through metabolic changes were studied by NMR-based metabolic profiling [132]. Plants produce many primary and secondary metabolites during stress. Two different options can define this process: osmotic adjustment, where the plant regulates solute synthesis to decrease the osmotic potential under adverse situations [133], and solute accumulation, which occurs due to reductions, interruptions or fluctuations in the solute transport [134]. Generally, osmotica are easily synthesised from readily accessible antecedents and are transformed to metabolically active and movable compounds. Overall, pre-existing metabolomic resources for legume research will benefit faba bean improvements. However, accelerated applications of these tools may uncover new solutes/biochemical markers for stress tolerance in faba bean breeding.

4.1. Metabolite Accumulation in Legumes in Response to Drought and Their Use as Biomarkers

4.1.1. Amino Acids

The accumulation of amino acids and other nitrogen-based molecules in response to stress is one of the most important biochemical signals for almost all plants. These essential metabolites are shown to accumulate during stresses in different legumes, including the model species *M. truncatula* and soybeans [135–139]. The accumulated enhanced protein breakdown is triggered by stress, and a higher amino acid concentration helps a plant to survive under stress [140].

Proline is found to be the most prevalent amino acid found in plant tissues under stress conditions (drought, cold and salinity). Singh et al. [141] demonstrated a correlation between drought and an increased free proline accumulation in drought-tolerant barley cultivars compared to a more drought-susceptible genotype. Similar correlations have been found in other crops [142], but there are also reports of poor correlations between drought and proline accumulations [143] in legume species. For example, *Lotus* spp. and soybeans showed a positive association with increased proline concentrations during the reproductive stage only [132,144]. Again, during water deficit, proline accumulation was observed in the pre-flowering stage of peanuts (*Arachis hypogaea* L.) [145]. Proline accumulation relies not only on the water status but, also, on the growth stage of the plant. Short-term stress can be identified through proline quantification. Venekamp et al. [146] found that just one day of water deficit at the seedling stage induced proline accumulation. An increased proline concentration in faba beans was observed with the increase of the stress intensity, and the variation in proline concentrations at the genotypic level was reported to be low under optimal conditions [46,107,147,148]. However, an exogenous proline application can decrease stomatal opening under drought, and this had a positive impact on drought tolerance mechanisms [149,150]. Proline accumulation provides an indication of the plant physiological status, i.e., whether it is stressed or not, but not a descriptive drought tolerance indicator in faba beans. An exogenous application of β-aminobutyric acid (BABA) as a nonprotein amino acid was shown to enhance the accumulation of proline and soluble sugars, which improved drought adaptation in faba beans [151].

Besides proline accumulation, other amino acids are also observed in response to droughts in plants [152]. In lentils, methionine, isoleucine, valine, arginine and histidine increased under drought stress [138]. Drought stress-mediated GABA (gamma-aminobutyric acid) synthesis was observed in *Arabidopsis thaliana*, soybeans and common beans [153]. Abiotic stresses enhanced cytosolic Ca^{2+} levels, which ultimately promoted GABA synthesis, but until now, no evidence was found where GABA stimulated the stomata opening or closing through its ion transporter function [154].

4.1.2. Polyamines

Another plant response to abiotic stresses is polyamines (PA) synthesis, but the association between PA accumulation and fortification remains inconclusive. Three commonly found polyamines are putrescine, spermidine and spermine. Usually, stress-tolerant accessions contain a higher amount of PA than sensitive accessions [155,156]. Nevertheless, all three PAs do not accumulate at the same time, but one type of PA shows a stronger response. For example, under PEG-osmotic stress conditions, lentil cotyledons and roots exhibited increased putrescine and spermidine, while the cadaverine presence was nonsignificant. Drought-adapted yellow lupin (*L. luteus* L.) accessions produced a high amount of PAs compared to moderately adapted genotypes. Drought stress also increased the concentrations of spermidine and spermine [156]. Beside leaves, seeds commonly exhibit opposite trends, where leaves accumulate more Pas, and the seed content is reduced. Nayyar et al. [157] observed more PA accumulations in chickpeas compared to soybeans under water-deficit conditions. In relay cropping with legumes, 18 rice (*Oryza sativa* L.) cultivars showed significantly higher spermine levels when exposed under salt stress [158]. With this information, it can be said that PA accumulation does not depend on a single factor. Multiple factors like plant species, stress type and level, and the physiological status of the studied tissues, are involved. The PA dynamics of abiotic stress effects can unveil contradictory results of PA accumulation. The stress tolerance of a given species often correlates with the PA pool size, which can answer some underlying questions about the importance of PAs in assisting the protection against stresses. In faba beans, spermine, cadaverine and total polyamine increased in parallel with the salinity level, whereas the putrescine and spermidine levels decreased [159]. In faba beans, PA accumulations in response to drought stress have not been studied to date.

4.1.3. Organic Acids

Organic acid plays an important role in plant metabolism as an early source of photosynthesis and precursors of the synthesis of many other compounds. Organic acids support stomata regulation, ion equilibrium, ammonium manufacture and nutrient absorption [160]. Among the profiled metabolite classes estimated from soybean leaf tissue, organic acids occupy around 20% of the metabolites [161]. Nitrogen fixation in the nodule is impacted by the malic acid concentration, which regulates the bacteroid respiration [162]. The abundance of organic acids also depends on the type of organ. Organic acid concentrations were lower in lentil root tissue than in cotyledon tissue [138]. Sassi et al. [163] observed a reduced amount of organic acids in common bean leaves under osmotic stress conditions. The amount of succinate was doubled in drought-treated soybean leaves. In lentils, isocitric, nicotinic and oxaloacetic acid concentrations increased considerably in plants exposed to osmotic stress [164]. In soybeans, reduced amounts of organic acids in drought-adapted genotypes suggested metabolic flexibility, as plants shifted from growth to survival [132]. Organic acid functions in response to stress in faba beans have not yet been demonstrated, but it could become an area for future enquiry.

4.1.4. Carbohydrates

Carbohydrates are often referred to as appropriate osmotica and represent one of the main components of osmoregulation in many plant species [165]. The metabolic profiling of the model forage legume *Lotus japonicus* L. when affected by drought showed the accumulation of the sugars fructose, galactose, glucose and maltose, while arabitol, ununitol and galactitol were the most abundant polyols [144]. In soybean leaves, about 30% of the metabolites identified were soluble sugars and sugar alcohols, which are important for plant adaptation against stresses, especially during water deficits [161]. The close association among carbohydrates and their involvement in the primary metabolism suggested a molecular modification may be possible to improve the stress tolerance through the synthesis and degradation of these compounds [166]. However, there is no general acceptance of the hypothesis that "osmotic adjustment arises from the accumulation of solute due to the persistence of photosynthesis after cessation of leaf growth" [133].

Among the dominant carbohydrates, trehalose, a nonreducing disaccharide, accumulates in large quantities in the unusual desiccation-tolerant plant *Myrothamnus flabellifolia* Wolw. [167,168]. High levels of trehalose can stabilise proteins and membranes [169]. It has been recently reported that rhizobium inoculation can also increase the dehydration tolerance of several legume species through trehalose biosynthesis [170–173]. Moreover, an increased trehalose concentration was found to be a significant contributor to drought tolerance [174]. Polyhydric alcohols or polyols are a major metabolite group reviewed by Merchant and Richter [175]. The quantity of polyols found in plant tissues was found to be sensitive to environmental conditions [176,177]. Further, Dumschott et al. [178] characterised D-pinitol as being influenced by the developmental stage. D-Pinitol is present in most legumes at some level under optimal conditions, but an increase is triggered during water and salinity stress [179]. While there is a general consensus on the function of pinitol in plant systems, gaps remain in understanding the conditions leading to pinitol accumulation, as well as the molecular control of the accumulation.

5. Faba Bean Genetic Resource to Aid Breeding for Drought Adaption

A wealth of faba germplasm is available, comprising over 43,000 accessions (Dr. Kenneth Street, personal communication) that are conserved mostly within 37 global gene banks. The ICARDA (International Centre for Agricultural Research in the Dry Areas) collection exceeds 9000 accessions (21% of the global collection) [55]. Wide genetic variations have already been reported in faba bean genetic resources for various traits, including physiological traits related to drought adaptation (reviewed in [82]). This enormous variability is expected in this ancient domesticated crop with its wide adaptability to various latitudes and altitudes. The rich germplasm collection of faba beans

should be systematically maintained and could be characterised for the physiological and metabolic parameters in order to improve drought adaptation in this species.

6. Conclusions

Improvement of global faba bean production will have a positive impact on the food supply and cropping systems of many nations. Tools to improve faba bean production will incorporate both management activities and improved genotypes. The adoption of these tools must balance the necessity for broad-scale characterisations and cost with the precision required for appropriate detection at the plant scale. Many traits have been identified for drought tolerance studies. Some traits, like canopy temperature, can be used efficiently for large numbers of genotypes in breeding programs, while, for example, carbon isotope discrimination is largely limited to low numbers of genotypes. Metabolite-based biomarkers and bioindicators can easily supplement the selection process in faba bean breeding programs. The identification and quantification of given primary and/or secondary metabolites against specific stresses could become valuable decision-making tools for plant breeders if their associations are of sufficient strength and their analytical costs are low. The available metabolomic resources from other legumes should be explored to understand the molecular insights of stresses. The large genome of the faba bean is currently being sequenced, which will soon make molecular breeding possible. Finally, no single trait and approach are adequate to improve yields under drought conditions, the most complex environmental factor for faba bean productivity. A combination of screening methods suitable for specific environments and expansion of the scale of breeding will be required to allow expansion of the production area and yield of the faba bean, a crop that is an increasingly important source of plant-based protein in drought-prone production regions.

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